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AWARD NUMBER: W81XWH-05-1-0181

TITLE: Activation and Protection of Dendritic Cells in the Prostate Cancer Environment

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REPORT DATE: January 2011

TYPE OF REPORT: Final Addendum

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
1 January 2011	Final Addendum	15 Sep 2010 – 14 Dec 2010

4. TITLE AND SUBTITLE

Activation and Protection of Dendritic Cells in the Prostate Cancer Environment

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-05-1-0181

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Virginia Commonwealth University
Richmond, VA 23298

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Final Report for research performed at the UMDNJ and VCU. Experiments have demonstrated for the first time the presence of endothelin receptors on murine dendritic cells (DC), and the fact of endothelin-1 production by murine DC upon stimulation with TNF• and lipopolysaccharide (LPS). The modification of the endothelin axis on DC changed its resistance against prostate cancer induced apoptosis – the blockade of ETA receptors resulted in the increased apoptotic rate, while the blockade of the ETB receptors lead to the increased survival of DC in the prostate cancer environment. Blockade of ETA receptors also resulted in decreased DC capability to express costimulatory molecules and promote T cell proliferation. Based on these data, in vivo experiments were carried out, in which mice with prostate cancer (RM1 cells) were treated with intratumoral injection of modified DC (stimulated DC, with ETB receptor blocked). This treatment resulted in reduction of prostate cancer growth in mice in the experimental group, in comparison to untreated control mice. Blockade of ETB receptors and pulsing of DC with tumor antigen also led to the reduction of tumor growth after subcutaneous injection of this vaccine. Studies are under way to elicit the mechanisms of endothelin axis action on DC, as well as the underlying mechanisms of interaction between DC and prostate cancer cells.

15. SUBJECT TERMS
Prostate cancer, Dendritic cells, Endothelin receptors, Immunotherapy

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
U	U	U	UU		USAMRMC

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Introduction:

This study is being conducted for the (i) characterization of the prostate cancer and dendritic cells (DC) interaction; (ii) defining the role of endothelin axis in the maturation of DC, (iii) elucidating the role of endothelin axis in the prostate cancer-DC interaction, and (iv) modification of dendritic cells to be used in the treatment of prostate cancer. Mouse model was used. This is the addendum to the final report of the Award, from September 15 2010 to December 15 2010, covering mainly gene array experiments performed at the Virginia Commonwealth University (VCU) School of Medicine. Only experiments performed during these 3 months are described. Addendums and references are removed. I kept *Key Research Accomplishments, Reportable Outcomes and Conclusions* sections from the final report, with minor modifications to account for the results of these recent experiments.

Body of the Report:

Gene array experiments were performed on dendritic cells using The GeneChip® Mouse Genome 430 2.0 Arrays from Affimetrix (Santa Clara, CA). Arrays include 45,000 probe sets that allow to analyze the expression level of over 39,000 transcripts and variants from over 34,000 well characterized mouse genes.

Eight conditions of dendritic cells were analyzed. These conditions include: 1) DC; 2) DC+TNFa; 3) DC+TNFa+BQ123 (endothelin A receptor inhibitor); 4) DC+TNFa+BQ788 (endothelin B receptor inhibitor); 5) DC+RM-1; 6) DC+RM-1 +TNFa; 7) DC+TNFa+BQ123+RM-1; 8) DC+TNFa+BQ788+RM-1; RNA derived from splenocytes provided control.

For this experiment, DC were cultured as described before, and TNFa and BQ123 or BQ788 were added for the last 48 hours. For RM-1 co-incubation, 7-day-old cultured DC were harvested and co-incubated with the murine prostate cancer cell line RM-1 in six-well plates for 48 hours. DC and tumor cells were separated using membrane inserts with 0.4-µm pore size, which exclude direct cell-to-cell contact, but allow free exchange of soluble factors. Specifically, 5×10^5 DC were placed in six-well plates in 3 ml of medium. One million prostate cancer cells resuspended in 2 ml of medium were placed into the inserts on the top of each well. Inserts with RM-1 cells were discarded and DC were harvested 48 h later, washed, RNA was extracted using RNA extraction minikit, and used for gene arrays. Gene arrays were performed as per manufacturer's recommendations. While comparing different conditions, we discarded genes which were not reliably expressed in all sets. Difference in gene expression deemed significant if change was twofold or higher.

While comparing DC cultured without intervention to DC incubated with RM-1 cells (groups 1 and 5), ppreliminary analyze of data demonstrated decreased expression of receptors for IL-12 and interferon gamma in DC incubated with RM-1 cells. In addition, there was increased expression in programmed cell death gene (difference - x2.11) in these DC as well. Incubation also led to the significant decrease in the heat

shock proteins expression, that might have reduced DC ability to withstand stress and anoxia.

Obviously, conducted experiments (gene arrays) contain vast amount of raw data and that cannot be analyzed in this limited space or time, and we will use these data for the future planning of the experiments. This time, we paid special attention to the influence of endothelin B receptor (ET_B) blockade on DC condition.
Comparison of groups 2 and 4 are provided below:

Table 1. Comparison of DC+TNFa to DC+TNFa+BQ788 - change of some genes expression:

Gene title	rate of change DC+TNFa+BQ788 / DC+TNFa
Interleukin 15	2.07
Interleukin 1α	2.2
Interleukin 6	2.37
Interleukin 18 receptor 1	2.78
X-linked inhibitor of apoptosis	2.29
Apoptosis inhibitor 5	3.45
Apoptosis regulator	3.64
Interleukin 1β	0.49
Interleukin 7 receptor	0.34
Interleukin 10 receptor	0.28
Programmed cell death 6 integrating protein	0.27
BCL2-like11 Apoptosis facilitator	0.48
Caspase 8	0.47

As it can be seen from provided table, the blockade of ET_B receptor induced increased expression of pro-inflammatory cytokines and some of the apoptosis inhibitors, while there was a decrease in the expression of pro-apoptotic genes.

As it can be seen from the list of compared conditions, we assessed the influence of endothelin receptor inhibitors on DC incubated with RM-1 cells.

The blockade of endothelin A receptors (ET_A) on DC incubated with RM-1 cells led to increased expression of the apoptosis inducing factor SIVA1 (x2.17), and apoptosis regulatory factor as well (x2.58).

Table 2 below demonstrates changes induced by the blockade of the ET_B receptors on DC incubated with RM-1 cells.

Table 2. DC+TNFα+RM-1 vs DC+TNFα+RM-1+BQ788

Gene title	rate of change DC+TNFa+RM-1+BQ788 / DC+TNFa+RM-1
X-linked inhibitor of apoptosis	2.57
Apoptosis inhibitor 5	4.30
Apoptosis regulator	6.80
Interleukin 18 receptor 1	2.25
Interleukin 15	2.47
Interleukin 18	2.94
Interleukin 15 receptor alpha chain	2.96
Interleukin 7 receptor	4.06
Interferon activating gene	11.92
Mature T cell proliferation	11.04
Death associated protein	0.29
MAP kinase activating death domain	0.44
Programmed cell death 2 like	0.41
Programmed cell death 1, ligand 2	0.29
Serine / Threonine kinase 17β, apoptosis inducing	0.31
SIVA 1 apoptosis inducing factor	0.22
Death associated protein 3	0.20
Caspase 4	0.15
Interleukin 13 receptor α1	0.13
Interleukin 10 receptor β	0.19
THAP domain containing apoptosis associated protein 2	0.23

As it can be seen from Table 2, modification of DC by blocking ET_B receptor in the cancer environment improves DC chances for survival (increased expression of apoptosis inhibitor genes). And increased expression of pro-inflammatory cytokines should render DC more capable of stimulating antitumor immune response. These features of ET_B receptor inhibitors should be very useful in their possible use in the clinical trials.

Key research Accomplishments:

- Production of ET-1 by murine DC has been documented first time, as well as the presence of endothelin receptors on murine DC upon their activation.

- The influence of endothelin receptor inhibitors on DC phenotype was demonstrated. Functional experiments (MLR) showed the possible involvement of the ET_A receptors in the activation of DC, driving them towards TH1 response. It seems that ET_B receptor stimulation might drive DC toward tolerance, with decreased expression of co-stimulatory molecules. Further studies are needed to clarify the exact role of these receptors in DC biology. Molecular biology experiments are scheduled to clarify the mechanisms of endothelin axis function in DC.

- We have demonstrated for the first time that the modification of endothelin axis on dendritic cells may result in increased resistance and improved survival against prostate cancer cells.

- Treatment of murine prostate cancer by intratumoral injection of the modified dendritic cells resulted in the reduction of the tumor growth. So did the treatment with subcutaneous injection of activated and antigen-pulsed DC. These data may provide basis for the development of clinical trials protocol.

- It has been shown first time that the modification of endothelin axis on dendritic cells (blockade of ET_A receptors) might alter immune response and prolong graft survival.

Reportable outcome:

Research data have been presented at the Annual meetings of the American Association for Cancer Research (AACR), and American Urological Association (AUA), as well as at the IMPaCT Meeting (September 5-8, 2007, Atlanta, GA). The Abstract of the presentation at the AUA is attached. Research data will be reported to the CITIM-2011 International conference in Budapest, Hungary as well (In May, 2011). Manuscript describing the major findings of the conducted research is in preparation and will be submitted in several weeks.

Conclusion:

Prostate cancer is the most common cancer in American men, and more than 32,000 are expected to die from it in this year [6]. There is now curative treatment once disease gets beyond prostate. Hormonal therapy, though efficient, is temporary and not curative, and its principle has not been changed for more than 50 years. Chemotherapy also provides only temporary and short-lived effect. Because of this, the search of alternative treatment options are vitally required. Immunotherapy based on antigen presenting cells did provide some relief to patients with hormone-resistant prostate cancer [7]. However, there is still a long way to go for it to become really effective, and large amount of work needs to be done to elucidate the mechanisms of immuneotherapy and make it efficient. Our experiments have demonstrated the possible role of endothelin receptor inhibitors in the function of DC, which can be useful in the treatment of different diseases, ranging from cancer to transplantation. Our in vivo experiments showed the effectiveness of endothelin axis modification on DC in the treatment of prostate cancer in mice. More experiments are scheduled to elucidate the finer mechanisms of DC-prostate cancer interaction, and clinical trials protocol is being planned for patients with advanced prostate cancer, using modified autologous DC. Final gene arrays data were very interesting in that it showed a lot of changes in DC favoring its survival with the modification of the ET_B receptor. We are planning to study the molecular mechanisms of ETB receptor's modification and its full effect on DC function and host's immune response.